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Upregulation of the TGFβ signalling pathway by Bcr-Abl: Implications for haemopoietic cell growth and chronic myeloid leukaemia

Gigi M.O. Møller[a], Victoria Frost[a], Junia V. Melo[b], Andrew Chantry[a,*]

[a] School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK
[b] Department of Haematology, Faculty of Medicine, Imperial College of Science, Technology and Medicine, Hammersmith Hospital, London W12 ONN, UK

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Abstract **Chronic myeloid leukaemia (CML) is a myeloproliferative disorder characterized by uncontrolled growth of progenitor cells expressing the tyrosine kinase fusion gene product, Bcr-Abl. At present, little is known regarding how TGFβ, and downstream Smad transcription factors, influence CML cell proliferation in the context of Bcr-Abl expression. Here we show that ectopic Bcr-Abl expression dramatically increases TGFβ/Smad-dependent transcriptional activity in Cosl cells, and that this may be due to enhancement of Smad promoter activity. Bcr-Abl expressing TF-1 myeloid cells are more potently growth arrested by TGFβ compared to the parental TF-1 cell line. Additionally, growth of Bcr-Abl-expressing CD34+ cells from chronic phase CML patients is inhibited by TGFβ and, interestingly, treatment of a non-proliferating CD34+ CML cell subpopulation with the TGFβ kinase inhibitor SB431542 enhanced cell death mediated by the Bcr-Abl inhibitor imatinib. Our data suggest that the expression of Bcr-Abl leads to hyper-responsiveness of myeloid cells to TGFβ, and we hypothesise that this novel cross-regulatory mechanism might play an important role in maintaining the transformed progenitor cell population in CML.**

Keywords: TGFβ; Smad; Bcr-Abl; Leukaemia

1. Introduction

TGFβ plays a vital role in maintaining the growth and differentiation balance in haemopoietic cells [1,2]. Its specific influence depends upon the cellular environment and differentiation status. In general, TGFβ is considered as a strong inhibitor of committed progenitor cell function, and autocrine production of TGFβ maintains haemopoietic stem cells in a quiescent state [3]. The intracellular components that transduce TGFβ signals into the nucleus have been unveiled in recent years (reviewed in [4]). Smad transcription factors act downstream of transmembrane serine–threonine kinase TGFβ receptors. To date, eight members of the Smad family have been described that can be segregated into three functionally distinct sub-groups. Upon activation, the TGFβ receptor complex induces phosphorylation of R-Smads (Smads 1, 2, 3, 5, 8), which then associate with a common-mediator co-Smad (Smad4). These heteromeric complexes are translocated to the nucleus, where they regulate gene transcription by association with DNA-binding proteins or direct binding to promoters for target genes.

Despite the wealth of recent data on the molecular details of TGFβ signalling, little is known regarding how TGFβ and Smads influence chronic myeloid leukaemia (CML) cell proliferation, although disruption of components within the TGFβ signalling cascade is known to drive many malignancies of haemopoietic origin [1]. CML is an example of a myeloproliferative disorder characterized by the uncontrolled clonal proliferation of transformed multipotent haemopoietic progenitor cells. The disease usually evolves in three stages: the chronic phase (CP), accelerated phase and blast crisis. Current treatments, which include a variety of chemotherapeutic drugs, stem cell transplantation, and more recently the Bcr-Abl kinase inhibitor imatinib [5], are usually only effective during the CP. The complete eradication of CML using imatinib is, however, hindered by a small pocket of resistant haemopoietic stem cells that lead to disease persistance [6]. CML was the first type of leukaemia to be associated with a specific chromosomal abnormality known as the Philadelphia (Ph) chromosome. This is the consequence of a t(9;22)(q34;ql l) chromosomal translocation which fuses part of the *ABL* proto-oncogene with the *BCR* gene. The product of this translocation is the constitutively active Bcr-Abl kinase [7], and Bcr-Abl leads to the malignant transformation of haemopoietic cells through several mechanisms linked to its activation of mitogenic signalling pathways [8].

In this study, we investigated the effects of Bcr-Abl expression on Smad/TGFβ signalling activity. We show that Bcr-Abl dramatically upregulates TGFβ signalling when expressed in Cosl cells via a mechanism that may involve enhancement of Smad promoter activity. We also show that TGFβ is a more potent growth inhibitor of Bcr-Abl expressing TF-1 myeloid cells compared to parental TF-1 cells. In addition, CD34+ cells isolated from CP-CML patients are growth arrested by TGFβ, and are more susceptible to imatinib-mediated cell death following inhibition of the TGFβ receptor kinase. Overall, our findings define a new cross-regulatory mechanism that can influence the growth of Bcr-Abl expressing haemopoietic cells, and we speculate that combined treatment with both imatinib and TGFβ kinase inhibitors could provide added therapeutic benefits in CML patients.

*Corresponding author. Fax: +44 1603 592250.
E-mail address: a.chantry@uea.ac.uk (A. Chantry).

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2. Materials and Methods

2.1. Cell Culture and growth assays

CD34+ cells from three CP-CML patients were selected from leukapheresis samples by binding to immunomagnetic beads (MiniMACS, Miltenyi Biotech, Bergisch-Gerbach, Germany), and maintained in RPMI-1640 medium with L-glutamine (Invitrogen), penicillin/streptomycin (Sigma) and 10% fetal bovine serum (Sigma). The purity of the CD34+ fraction after immunomagnetic selection was 94–98%, and 2×10^5 cells used routinely in each experiment. Cell fractionation was performed on fresh leukapheresis samples, and the CD34+ cells were cryopreserved in DMSO/FCS at −180 °C in aliquots of 10^5–10^6 cells per vial, until thawed for the experiment. All three patients used in this study were newly diagnosed as CML in chronic phase, with WBC in the $160–235 \times 10^9$/L range, and none had received imatinib or any other form of chemotherapy prior to leukapheresis. All three patients had baseline level of BCR-ABL transcripts and 100% Ph+ metaphases. Although FISH was not performed, our experience from a large number of patients who have very high WBC counts at diagnosis is that their CD34+ fraction contains <3%, if any, *BCR-ABL*-negative cells.

TF-1 cells expressing Bcr-Abl-GFP were generated using protocols described previously [9], and maintained in RPMI-1640 medium with L-glutamine (Invitrogen), penicillin/streptomycin (Sigma) and 10% fetal bovine serum (Sigma) together with 5 ng/ml GM-CSF. In some experiments, CP-CML cells were grown in the presence of the following: 1 ng/mL recombinant human interleukin-1β (rhIL-1β) and 50 ng/mL of rhIL-6, rhIL-3 and stem cell factor (hrSCF). Cos-1 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cell growth curves were obtained using a haematocytometer and non-viable cells excluded with trypan blue. Growth of CD34 positive cells from CP-CML patients was assessed using an automated cell counter (SYSMEX). HEK-293 cells were transfected with Smad2-HA expression construct as described [10].

2.2. Plasmids, reagents, and antibodies

Bcr-Abl p210 cloned into a pXMT2 expression vector was obtained from Dr. Helen James (University of East Anglia, UK), CAGA12 from Caroline Hill (CRUK Laboratories, London, UK), pGL3-Smad2P from Koichi Hagiwara (Saitama Medical School, Japan), and Smad3P-luc from Thomas Kelley (Case Western Reserve University, USA). These plasmids were used for transient transfection of Cos-1 cells using Fugene (Roche). Imatinib mesylate (STI571, Gleevec) was obtained from Novartis Pharmaceuticals (Basel, Switzerland), and SB431542 (TGFβ-RI inhibitor) from Tocris Ltd. Antibodies used included anti-phosphotyrosine (Sigma), anti-Smad2 and Smad3 (BD transduction laboratories), anti-β-actin (Sigma), anti-Abl (Calbiochem). Secondary antibodies were HRP-conjugated goat anti-rabbit or goat anti-mouse (Sigma).

2.3. Luciferase assay

Smad responsive reporter plasmid CAGA12 (2 μg) together with cDNA constructs encoding Bcr-Abl (2 μg) and Renilla control plasmid were transfected into Cos-1 cells in Dulbecco's modified Eagle's medium with 0.5% fetal bovine serum using Fugene 6. Cells were stimulated with TGFβ1, and the transcriptional activity of the reporter plasmid monitored by luciferase measurements (Roche). Smad promoter activity was assessed by transfecting Cos-1 cells with 2 μg of pGL3-Smad2P vector containing a 3030-bp fragment of the human Smad2 promoter [11], or Smad3P-luc containing 1892 bp of the human Smad3 promoter [12]. Empty pGL3 vector was used as a negative control. Each set of experiments was performed at least three times and the results show the mean of the experiments with standard error bars.

2.4. Cell lysate preparation and Western blots

Total cell extracts were obtained by lysing $0.8–1 \times 10^7$ cells in 150 μL of buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol, and protease and phosphatase inhibitors). Lysates were cleared by centrifugation at 12000 rpm and supernatant was isolated and stored at −20 °C. Normally, 100 μg of protein was separated by SDS–PAGE and electroblotted onto nitrocellulose membrane. Membranes were blocked in 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS), and then incubated overnight in the same buffer containing the primary antibody. Following incubation with appropriate secondary antibodies, membranes were visualised using ECL reagent (Amersham).

2.5. FACS analysis

Cell cycle status was assessed following the staining of cells with propidium iodide (PI) and flow cytometry analysis. Aliquots of 2×10^5 cells were washed in PBS and placed in 50% ice-cold ethanol for 30 min. After three PBS washes, cells were incubated with 100 μg/mL PI/RNAse mix at room temperature in the dark for at least 1 h. Stained cells were counted on a FACScan flow cytometer (Becton Dickinson), and the data obtained were analysed using WinMDI and Cylchred. For determination of CD34 expression, aliquots of 2×10^5 cells were stained with 1 μL of CD34-PerCP antibody (Becton Dickinson). After 30 min at 4 °C in the dark, cells were washed twice in PBS. Isotype-matched antibodies were used as negative controls to establish the background fluorescence of each sample. Stained cells were analysed using a FACScan flow cytometer (Becton Dickinson), and the data obtained were analysed using WinMDI.

3. Results

3.1. Bcr-Abl upregulates the transcriptional activity of a Smad responsive reporter plasmid

Initially, we investigated potential cross-talk between the TGFβ signalling pathway and Bcr-Abl in Cosl cells, an epithelial cell line that has been routinely been used to study TGFβ-dependent signalling activity. Cos-1 cells were transfected with a Bcr-Abl expression plasmid together with a TGFβ/Smad-responsive luciferase reporter plasmid known as CAGA12, which contains 12 tandem copies of a Smad/DNA-binding element. Cells were stimulated with increasing amounts of TGFβ, and the luciferase activity in cell lysates was monitored as a measure of the transcriptional activity of the CAGA12 reporter plasmid. There was a significant, approximately 30-fold with Bcr-Abl vs. 12-fold w/o Bcr-Abl, concentration-dependent synergistic activation of CAGA12 in the presence of both TGFβ1 and Bcr-Abl that peaked around 5 ng/mL TGFβ (Fig. 1A). We then examined the effect of specific inhibitors on the synergistic CAGA12 activation in the presence of Bcr-Abl and TGFβ. Cos-1 cells transfected with CAGA12 and Bcr-Abl in the presence or absence of TGFβ were treated with 10 μM of imatinib for 2 h prior to TGFβ stimulation. The synergistic activation of the transcriptional activity of CAGA12 obtained with Bcr-Abl and TGFβ was partially inhibited by imatinib (8-fold increase in presence of imatinib compared to 25-fold in controls), even though Bcr-Abl kinase activity was completely inhibited based on phosphotyrosine Western blotting (Fig. 1B). This suggests that the influence of Bcr-Abl on the TGFβ signalling cascade is not exclusively mediated by its' kinase domain, although we cannot exclude the possibility that there may be some residual Bcr-Abl kinase activity that is not detected by anti-phosphotyrosine blotting. SB431542 is a potent and selective inhibitor of the TGFβ type I receptor. Cos-1 cells transfected with CAGA12 and Bcr-Abl, in the presence or absence of TGFβ1, were treated with 10 μM of SB431542 for 2 h prior to TGFβ stimulation. In this instance, synergistic activation of the transcriptional activity of CAGA12 was completely inhibited by SB431542 which itself had no effect on Bcr-Abl kinase activity (Fig. 1B). These data suggest that the Bcr-Abl influenced upregulation of CAGA12 is mediated by the TGFβ type I receptor activation.

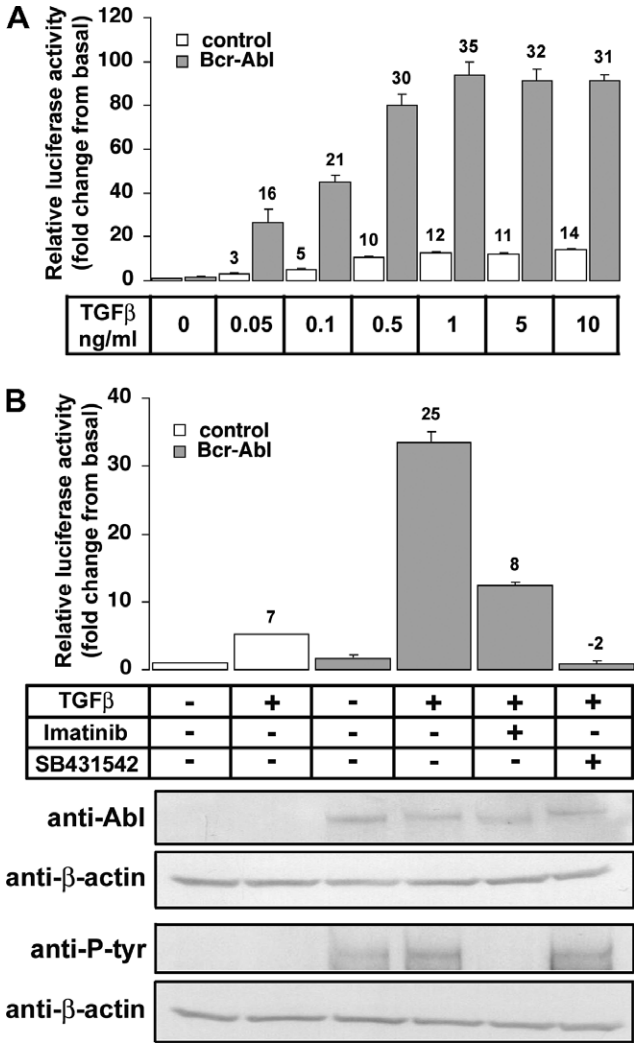

Fig. 1. Upregulation of TGFβ signalling by Bcr-Abl in transfected Cos-1 cells. Cos-1 cells were co-transfected with a Bcr-Abl expression plasmid or the corresponding empty vector together with a TGFβ/Smad-responsive CAGA12-luciferase reporter construct. (A) Cells were stimulated for 4 hours with varying concentrations of TGFβ1 (0–10 ng/ml), and the luciferase activity was monitored. Data were normalised to a control plasmid and are the average of at least three separate experiments ± S.E. The fold differences between non-Bcr-Abl expressing controls vs. TGFβ stimulated cells as well as Bcr-Abl expressing controls vs. TGFβ stimulated cells is indicated above the relevant bars. (B) Cells pre-stimulated for 2 h with either 10 μM of imatinib, 10 μM of SB431542 or 0.1% DMSO. 5 ng/ml of TGFβ1 was then added to the culture media for 4 h and the luciferase activity was determined. Data were normalised to a control plasmid and are the average of at least three separate experiments ± S.E. The fold differences between non-Bcr-Abl expressing controls vs. TGFβ stimulated cells as well as Bcr-Abl expressing controls vs. TGFβ stimulated cells is indicated above the relevant bars. The bottom panel represents anti-Abl, anti-β-actin and anti-phosphotyrosine Western blots of cell lysates used in this experiment.

3.2. Bcr-Abl enhances Smad2 and Smad3 promoter activity in Cosl cells

Studies have shown previously that Bcr-Abl can upregulate gene subsets by targeting specific promoter elements [13]. In addition, the activity of important cell signalling regulators such as protein-tyrosine phosphatase IB (PTP IB) for example is directly controlled by Bcr-Abl enhancing PTP IB promoter activity [14]. To begin to investigate the mechanisms driving increased TGFβ signalling activity in the presence of Bcr-Abl, we initially examined the effect of Bcr-Abl on the promoter activity of receptor-regulated Smad2 and Smad3. Cos-1 cells were transfected with luciferase reporter plasmids containing a 3030 bp human Smad2 promoter fragment referred to as pGL3-Smad2P [11], or a 1892 bp fragment of the Smad3 promoter known as pGL3-Smad3P [12], together with increasing amounts of a Bcr-Abl expression plasmid. Luciferase assays showed a significant concentration-dependent upregulation of both the Smad2 and Smad3 promoters in the presence of Bcr-Abl, with no effect on the empty pGL3b vector (Fig. 2A and B). Increasing levels of Bcr-Abl expression in these experiments was also confirmed by anti-abl Western blotting (lower panels in Fig. 2A and B). Changes in Smad promoter activity in the presence of Bcr-Abl were also reflected in increases in endogenous Smad2 and Smad3 protein levels determined by Western blotting (Fig. 2C). These results suggest that one mechanism for enhanced TGFβ signalling activity in the presence of Bcr-Abl is by increasing the levels of Smad expression. Although we have also observed no significant alterations in rates of Smad translocation and Smad c-terminal phosphorylation in the presence of Bcr-Abl (data not shown), we cannot rule out additional cross-regulatory mechanisms.

3.3. TGFβ-induced growth arrest is enhanced in myeloid cells expressing Bcr-Abl and CD34 positive cells from CP-CML patients

Since expression of Bcr-Abl significantly enhanced TGFβ/Smad signalling activity in Cosl cells, we next examined the effects of TGFβ on TF-1 cells, a GM-CSF-dependent myeloid leukaemic cell line that has been established as a model system for CML blast crisis [15,16]. A TF-1 cell line stably expressing Bcr-Abl-GFP was generated as described previously for 32D cells [9], and Bcr-Abl expression confirmed by anti-Abl Western blotting (data not shown). Comparing the growth of parental TF-1 cells and TF-1/Bcr-Abl cells indicates that TGFβ has a greater growth inhibitory effect on cells expressing Bcr-Abl (Fig. 3A). After 7 days in culture, there are approximately 80% of TF-1 cells in TGFβ-stimulated relative to unstimulated controls compared to approximately 55% remaining in the TF-1/Bcr-Abl cells (Fig. 3A). Therefore, in a myeloid cell context, the presence of Bcr-Abl does indeed enhance cellular responsiveness to TGFβ. Next, in order to assess whether the TGFβ growth inhibitory response is active in primary CML cells, CD34 positive cells from CP-CML patients were cultured for 7 days with or without TGFβ, and also in the presence of the SB431542 TGFβ kinase inhibitor. Growth and viability were analysed at days 1, 2, 5 and 7. CP-CML progenitor cells were clearly susceptible to the growth inhibitory effect of TGFβ (Fig. 3B). Conversely, inhibition of endogenous TGFβ signalling using SB431542 caused a slight increase in cell growth compared to the un-stimulated control cells. PI staining of CP-CML CD34+ cells showed an increase in the G1 and a decrease in the S-phase sub-populations after 24 h TGFβ stimulation (Fig. 3C). Overall, these results indicate that TGFβ potently inhibits the growth CD34 positive cells from CP-CML patients expressing Bcr-Abl. However, due to the difficulties associated with obtaining CD34+ cells from non-CML patients for use as Bcr-Abl negative controls, we are

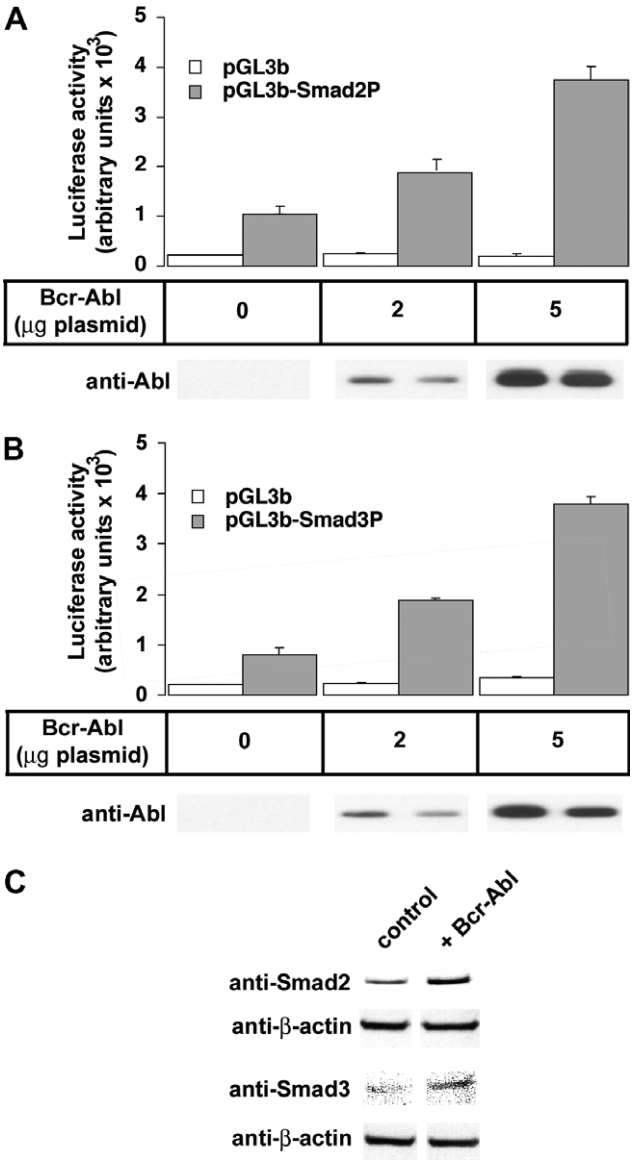

Fig. 2. Bcr-Abl increases Smad promoter activity and Smad expression. Cos-1 cells were co-transfected with 2 μg or 5 μg Bcr-Abl expression plasmid or the corresponding empty vector as indicated, together with either an empty pGL3 vector (pGL3b), the Smad2 (A) or Smad3 (B) promoter in a pGL3b luciferase reporter construct. Cells in these experiments were not treated with TGFβ, and the luciferase activity was determined 24 h after transfection. Data were normalised to a control plasmid and are the average of at least three experiments ± S.E. Expression of increasing amounts of Bcr-Abl was confirmed by anti-abl Western blots shown in (A) and (B) as separate panels below the graphs. (C) Samples from the above experiment transfected with 5 μg Bcr-Abl expression plasmid or an empty vector were Western blotted against anti-Smad2, anti-Smad3 or anti-β-actin antibodies.

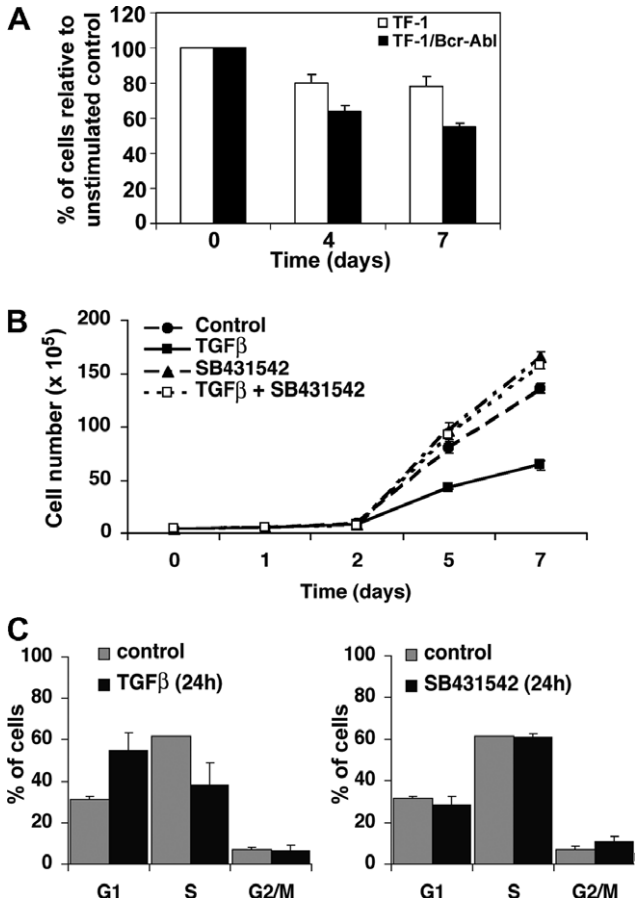

Fig. 3. TGFβ inhibits the growth of TF-1 myeloid cells more potently in the presence of Bcr-Abl and CD34+ cells isolated from CML patients. (A) TF-1 cells or TF-1/Bcr-Abl cells were grown in the presence absence of 5 ng/ml TGFβ for the times indicated. Data are expressed as percentage of cells remaining after TGFβ stimulation relative to unstimulated controls. Data are the average of triplicates performed in two separate experiments. (B) CD34 positive cells from CP-CML patients were grown in the presence of either 5 ng/ml of TGFβ1, 10 μM of SB431542 or a combination of both. Growth was assessed using an automated cell counter (SYSMEX). Data represent the average of triplicate experiments on three separate patients samples ± S.E. (C). Cell cycle analysis was performed following the staining of cells with PI followed by flow cytometry using a FACScan flow cytometer (Becton Dickinson), and the data obtained were analysed using WinMDI and Cylchred. Data are the average of triplicates performed on two separate patient samples.

not able to definitively show that TGFβ signalling is upregulated in the context of Bcr-Abl expression in CML.

3.4. Inhibition of the TGFβ receptor kinase potentiates imatinib-mediated cell death of CD34 positive cells from CP-CML patients

CD34 positive cells from CP-CML patients clearly undergo enhanced and sustained growth when maintained in the presence of added growth factors (Fig. 3B), and these proliferating cells are known to be acutely sensitive to the Bcr-Abl kinase inhibitor imatinib which preferentially targets dividing cells [5]. Interestingly, a small population of residual non-dividing progenitor cells persist in imatinib-treated CML patients, and these cells may be the prime cause of disease relapse following imatinib treatment [17,18]. We hypothesised that upregulation of the growth inhibitory response to TGFβ in CD34+ progenitor cells by Bcr-Abl might contribute to the maintenance of a non-dividing cell population that is refractory to imatinib-mediated cell death. Therefore, to begin to analyse this non-dividing cohort of cells, we studied the effects of growth factor withdrawal on CD34+ CP-CML cells by maintenance in media containing no added cytokines with the addition of fresh media at days 3, 5 and 7 of culture. Even in the absence of growth factors, more than 50% of the cells

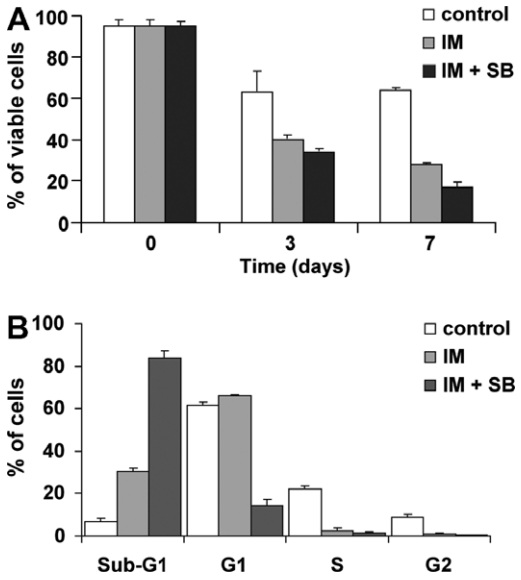

Fig. 4. Inhibition of TGFβ signalling in non-cycling CML CD34+ cells potentiates imatinib-mediated cell death. (A) CD34+ CP-CML cell viability was assessed by counting cells daily using a haemocytometer and identifying non-viable cells using trypan blue exclusion. Imatinib (1 μM) or Imatinib/SB431542 were added to cytokine free growth medium from Day 0 as indicated. (B) CD34 positive cells from CP-CML patients were grown in the presence of either 1 μM of imatinib alone or in conjunction with 10 μM of SB431542 for eight days. Cell cycle analysis was performed by staining of cells with PI and analysis with a FACScan flow cytometer (Becton Dickinson), and the data obtained were assessed using WinMDI and Cylchred. Data are the average of triplicate experiments performed on two separate patient samples.

remained viable within the first 7 days of culture (Fig. 4A), and it is likely that this subpopulation enters a dormant state, in which they exit from the cell cycle. After 7 days of exposure to imatinib approximately 30% of CD34+ CP-CML cells remained viable and the inclusion of the TGFβ receptor kinase inhibitor SB431542 reduced the number further to approximately 20% (Fig. 4A). Cell cycle analysis of the culture population at that time-point showed that imatinib led to an overwhelming accumulation of live cells in the sub-Gl phase of the cell cycle (Fig. 4B). However, combined treatment with both imatinib and SB431542 caused the number of dead cells in sub-Gl phase to increase dramatically with a concomitant reduction in the Gl sub-population (Fig. 4B). These results suggest that dual treatment of CD34+ CP-CML cells maintained in the absence of added cytokines with both imatinib and SB431542 is indeed more effective than imatinib treatment alone.

4. Discussion

The transforming growth factor-β (TGFβ) family of growth factors are important regulators of haemopoietic cell function, although individual cell lineages can respond positively or negatively depending upon their level of differentiation and maturation [1,2]. In general, TGFβ is considered as a strong inhibitor of committed progenitor cell growth and differentiation, and the autocrine production of TGFβ maintains immature CD34+ haemopoietic progenitor cells in a quiescent state. Interestingly, studies of haemopoietic pathologies and leukaemias have shown abnormalities in TGFβ signalling activity in both early myeloid leukaemia [19], and lymphocytic leukaemia [20]. In CML, the oncogenic Bcr-Abl fusion protein drives haemopoietic cell transformation, and also sustains a rare cohort of quiescent Bcr-Abl+ haemopoietic progenitor cells that persist throughout the CML-CP [21]. In this study, we explored connections between Bcr-Abl and TGFβ signal transduction pathway in model systems, haemopoietic cell lines and BcrAbl-expressing CD34+ cells isolated from CP-CML patients. Initially, we have shown that in TGFβ-responsive Cos-1 cells, the ectopic expression of Bcr-Abl significantly upregulates TGFβ mediated transcriptional activity. This phenomenon is mediated by the TGFβ type I receptor and partly by the kinase domain of Bcr-Abl. We then demonstrated that TGFβ has a significantly greater growth inhibitory effect on myeloid TF-1 cells expressing Bcr-Abl compared to the parental TF-1 cell line. In addition, TGFβ potently inhibits the growth of BcrAbl-expressing CD34+ cells isolated from CP-CML patients by inducing cell cycle arrest. Finally, we show that inhibition of the TGFβ receptor kinase in a quiescent sub-population of these Bcr-Abl-expressing CD34+ cells overcomes the cell cycle blockade by TGFβ, and enhances their sensitivity to cell death mediated by the Bcr-Abl kinase inhibitor imatinib.

We have shown for the first time that the expression of Bcr-Abl can act synergistically to upregulate TGFβ transcriptional activity in Cos-1 cells transfected with the TGFβ-responsive CAGA12 luciferase reporter. Initially, this was an unexpected result since several reports have demonstrated that TGFβ signalling and Smad functional activity is generally inhibited by intracellular signalling cascades downstream of receptor tyrosine kinases [22–25]. In these instances, the cross-talk mechanisms generally involve effects on Smad nuclear translocation. In our experiments, Bcr-Abl does not prevent TGFβ-induced movement of Smads into the nucleus. However, here we show that Bcr-Abl does increase the activity of both a 3030 bp fragment of the human Smad2 promoter, and an 1892 bp fragment of the Smad3 promoter, and also increases Smad2 and Smad3 protein expression. Therefore, the synergistic activation of TGFβ signalling activity in the presence of Bcr-Abl could be due to a direct effect of signalling pathways downstream of Bcr-Abl on Smad promoter activity, leading to increased Smad expression and enhanced activation of the TGFβ signalling pathway.

The final results presented investigate the more physiological aspect of TGFβ signalling in the context of CML. Strong inhibition of BcrAbl-expressing CD34+ cells isolated from CP-CML patients suggests that Bcr-Abl is not able to inhibit TGFβ signalling in a pathophysiological context. This response is likely to be cell type dependent, and this may explain why our data contrast with a recent report suggesting that in Ba/F3 and K562 cells, Bcr-Abl inhibits the cytostatic effect of TGFβ through the AKT/FoxO3 signalling pathway [26]. We have also observed that K562 cells are insensitive to TGFβ-mediated growth inhibition (data not shown). However, in our study, it is clear that CD34+ cells isolated from CML patients are highly responsive to the cytostatic action of TGFβ, even thought they express Bcr-Abl. In addition, we find that the expression of Bcr-Abl in a myeloid TF-1 cell background enhances their responsiveness to the TGFβ growth inhibitory activity.

In summary, our data as well as published work suggest that TGFβ is a strong inhibitor of haemopoietic cell growth and differentiation. The complete eradication of CML is also hindered by a small pocket of haemopoietic stem cells which are resistant to imatinib, in part because they are non-cycling [6]. Therefore, TGFβ is a prime candidate for maintaining these haemopoietic stem cells in a non-cycling state. Furthermore, we have demonstrated an upregulation or prolongation of TGFβ signalling by Bcr-Abl, which would suggest that one of the mechanisms by which Bcr-Abl promotes the transformation of haemopoietic progenitor cells, is by influencing the level of TGFβ signalling activity. We therefore believe that TGFβ could play a vital role in the preservation of the malignant progenitor population, and be partly responsible for the resistance to treatments targeting Bcr-Abl that is observed in a proportion of CML patients. Our findings are also interesting in light of recent mathematical models of CML which propose that combination treatment of imatinib with a cell cycle stimulant of progenitor cells could be effective in eliminating disease persistence in CML [27]. An important goal for the future is to study the effects of TGFβ on CML patient cells at different stages of differentiation to elucidate cross-regulatory mechanisms during chronic phase, accelerated phase and blast crisis.

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